

What is claimed is:

1. (original) A composition, comprising:  
a plurality of double-stranded RNA (dsRNA) fragments having  
overlapping sequences, each fragment having a size in the range of  
18-30 nt, wherein the composition is formed by enzymatic digestion of  
one or more large dsRNAs and wherein less than 2nM of the  
composition is capable of specifically silencing expression of a target  
gene by at least 65% in transfected COS cells.
2. (original) A composition according to claim 1, wherein the  
composition is capable of specifically silencing target gene expression  
by 70%.
3. (original) A composition according to claim 1, wherein the  
composition is capable of specifically silencing target gene expression  
by 80%.
4. (original) A composition according to claim 1, wherein the dsRNA  
has a size of at least 100 nt in length.
5. (original) A composition according to claim 1, wherein the plurality  
of fragments is at least 5 fragments.
6. (original) A composition according to claim 1, wherein the plurality  
of fragments is at least 10 fragments.
7. (original) A composition according to claim 1, wherein the large  
dsRNA has a sequence identity with a first portion of a messenger RNA

(mRNA) sequence such that the plurality of dsRNA fragments derived therefrom has a greater gene silencing activity at less than 2nM than a second plurality of fragments having sequence identity with a second portion of the mRNA.

8. (original) A composition according to claim 1, wherein the plurality of dsRNA fragments have a greater gene silencing activity at a concentration of less than 2nM than any single fragment in the composition.

9. (original) A composition according to claim 1, wherein the enzymatic digestion is achieved using RNaseIII in a manganese buffer or a mutant RNaseIII.

10. (original) A composition according to claim 1, wherein the target gene encodes Erk1 or Erk2 and the large dsRNA has sequence identity with a portion of mRNA transcribed from the gene.

11. (original) A composition according to claim 1, wherein the target gene encodes Ffluc or Renilla luciferase and the large dsRNA has sequence identity with a portion of mRNA transcribed from the gene.

12. (original) A composition according to claim 1, wherein the fragments are derived from digestion of a plurality of dsRNAs and wherein the plurality of dsRNA have sequence identity with non-contiguous regions of the mRNA.

13. (original) A composition according to claim 1, wherein the fragments are derived from digestion of a plurality of dsRNA wherein

the plurality of dsRNA has sequence identity with contiguous regions of the mRNA.

14. (original) A composition according to claim 1, wherein 1nM of the composition is capable of silencing gene expression by at least 70%.

15. (withdrawn) A method of preparing a composition described in claim 1, comprising:

(a) transcribing at least one RNA molecule having a sequence identity with a portion of a target gene, to form a large dsRNA;

(b) cleaving the large dsRNA into a mixture of overlapping fragments having a size in the range of 18-30 nt by means of RNaseIII or mutants thereof;

(c) determining whether less than 2nM of the large dsRNA can silence at 65% of gene expression of the target gene in COS cells after transfection; and

(d) obtaining the composition described in claim 1.

16. (withdrawn) A method of silencing gene expression; comprising:

(a) cleaving with an enzyme, a large dsRNA having sequence identity with a target gene, wherein the enzyme is RNaseIII or a mutant thereof and the cleavage product is a set of overlapping fragments of dsRNA in which greater than 80% of the fragments have a size of less than about 40nt;

(b) transfecting cells with the cleavage product of step (a) without size fractionating the product; and

(c) obtaining at least 65% silencing of expression of the target gene.

17. (withdrawn) A method according to claim 16, wherein step (b) further comprises: transfecting cells with less than 2nM of the cleavage product of step (a).

18. (withdrawn) A method according to claim 16, wherein RNase III cleavage is achieved in the presence of manganese buffer.